



Hybridization in the European carpenter ants *Camponotus herculeanus* and *C. ligniperda* (Hymenoptera: Formicidae)

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Abstract

The first case of hybridization between the large European carpenter ants *Camponotus herculeanus* (Linnaeus 1758) and *C. ligniperda* (Latreille 1802) is demonstrated by means of exploratory and hypothesis-driven data analyses of standardized phenotypic characters. The strong signal separating the parental species allows the identification of hybrid workers on the individual level, based on only seven characters. The frequency of hybridization between the two species is estimated for Central Europe as 0.2–1.0%. This low ratio indicates strong reproductive barriers considering syntopic occurrence at about 10% of the observation sites, a nearly complete overlap of swarming times and basically equal meteorological conditions to release swarming. The presented case increases the known ratio of hybridizing species within the 178 ant species of Central Europe to 19.1%. This figure dramatically contrasts the known hybridization ratio of 0.55% within an estimated number of 2000 ant species from the Holarctic outside Central Europe. This 30-fold lower discovery rate of hybrids may be explained by the predominance of idiosyncratic species delimitation methods in morphology-based taxonomy in combination with the psychology of human decision-making. A neotype of *Camponotus ligniperda* is fixed in a specimen from the *terra typica* and comments on the Latin naming are given.

Keywords Sister species · Hybridization · Allometry · Numeric taxonomy · Reproductive barrier

Introduction: on hybridization

Assuming that evolution of organisms takes place and that all species of plants and animals were not created by god as fixed entities some 6300 years ago, actively working taxonomists should expect to inevitably encounter hybrid specimens. However, many zootaxonomists, if asked for hybrid cases in the group of organisms which they study, answer that they do not know of hybrid cases, that hybridization is an extremely rare exception, or that it is not in the scope of their research. Such an evasive response was the regular result of an inquiry by the author in the year 2012 among colleagues doing taxonomic research in different groups of invertebrates such as Nematoda, Gamasidae, Oribatidae, Araneae, Collembola, Myriapoda, Odonata, Symphyta, Staphylinidae, and Carabidae. This low level of awareness appears surprising at first as the significant

function of hybridization or of horizontal gene transfer in the evolution of eumetazoons has been long known and is becoming increasingly recognized (e.g., Anderson & Stebbins 1954; Bullini 1994; Barton 2001; Abbott et al. 2013). This failure of taxonomists is explained by the prevailing of idiosyncratic species delimitation methods in combination with the psychology or economy of human decision-making. This idea comes from the every-day experience that we feel more comfortable, if not to say relieved, when having found clear YES/NO decisions rather than allowing undecided questions to circulate in our brain for longer periods. The latter is a stressful neuronal activity both in terms of real metabolic costs and of psychological discomfort. Translated to the situation of idiosyncratic taxonomy that does not use testable data sets obtained under defined standards, specimens of intermediate phenotype are comfortably interpreted as variations of a particular species rather than being supposed to represent hybrids. Yet, if we are inescapably nailed to the ground by the power of objective data, we feel forced to thoroughly investigate what is behind a disturbing observation.

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Hybridization in ants is widely spread within the Central European fauna. Excluding various successful laboratory crosses but including the outdoor population reported here, hybridization has been documented now in 34 of the 178 free living Central European ant species, in 25 different hybrid combinations, and in six genera (Pearson 1983; Seifert 1984; Fischer 1987; Douwes and Stille 1991; Seifert 1991; Seifert 1999; Seifert and Goropashnaya 2004; Pusch et al. 2006; Seifert 2006; Kulmuni et al. 2010; Bernasconi et al. 2010; Seifert et al. 2010; Steiner et al. 2010; Van der Have et al. 2011; Bagherian et al. 2012; Seifert 2013; Purcell et al. 2016). The identification of ant hybrids was performed in nine cases by Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) alone, in 14 cases by a combination of NUMOBAT and investigations of either nuDNA, mtDNA, isoenzymes, or caryotypes, and in one case each by nuDNA and isoenzyme analysis alone. These data add to a total of 19.1% of hybridizing species within the ant fauna of Central Europe.

The figures known from the Holarctic outside Central Europe differ dramatically. Hybridization is known here from 11 species of the genera *Acanthomyops*, *Messor*, *Pogonomyrmex*, and *Solenopsis*—this is only 0.55% within an estimated number of 2000 ant species. This 30-fold lower discovery rate of hybrids cannot reflect a real phenomenon, but is a consequence of the enormous dominance of idiosyncratic approaches in morphology-based ant taxonomy. It is obvious that idiosyncratically working ant taxonomists have a very low chance of becoming aware of hybridization cases and that they prevent themselves from approximating their knowledge to the real structure of biodiversity. This is illustrated by the fact that only the hybridization cases in *Acanthomyops* were credibly shown by a classical morphology-based taxonomist who, however, already used simple numeric methods of character description and processing (Wing 1968). Wing's hybrid indications were later confirmed by isoenzyme data (Umphrey and Danzmann 1998). Hybridization in *Solenopsis* was shown by data on genetics, biochemistry, and behavior (Ross et al. 1987, Vander Meer and Lofgren 1989) and hybridization in *Pogonomyrmex* by genetic data (Helms-Cahan et al. 2003). Suspicion of the *Messor* hybridization in Italy was raised by subjective taxonomic guessing and finally confirmed by a combination of NUMOBAT and of data on genetics and chorology (Steiner et al. 2011).

Hybrid identification by advanced methods of NUMOBAT is most effectively done within the vectorial space. The most powerful methods are here discriminant functions in which clearly defined samples of parental species form the basal vector-defining hypothesis, whereas suspicious samples are run as wild cards, i.e., without imposing a hypothesis. This method has been successfully used in *Myrmica*, *Lasius*, and *Formica* ants (Bagherian et al. 2012;

Kulmuni et al. 2010; Seifert 2006; Seifert et al. 2010) and in unpublished hybrid studies of the author in *Lasius*, *Formica*, and *Temnothorax*. Yet, there is no guarantee of success. Hybrid identification by NUMOBAT will fail if the involved parental species are too similar or, more rarely, if a hybrid of clearly separable parental species is likely to represent a phenotypic copy of a third species. Considering these drawbacks, I estimate that about 10% of 750 theoretically conceivable hybrid combinations within the fauna of Central Europe are not exposed by NUMOBAT data and require a thorough investigation by nuDNA markers or molecular expression products of nuclear DNA.

The taxonomy of the two largest ants of Central Europe, *Camponotus herculeanus* (Linnaeus, 1758) and *Camponotus ligniperda* (Latreille, 1802), has been subjected to many different interpretations. Observation of individuals with seemingly transitional pigmentation characters in areas where both species are in contact prompted Pisarski (1961) to suppose hybridization and to degrade *C. herculeanus* and *C. ligniperda* to subspecies status. Bernard (1968) went even farther in declaring full conspecificity, whereas Kutter (1977), though expressing some doubts, treated the two nomenclatorically as different species. The first distinguished statement in favor of heterospecificity was given by Seifert (1989) based on nonoverlapping NUMOBAT data after correction in the allometric space and a clearly differing ecology and life history. This conception and the underlying data were thoroughly confirmed by observations in the European range in the time since then (Seifert 2018) and every attempt to discover a hybrid of the two species failed over the now 40 years of my personal ant observation.

Yet, hybridization between *C. herculeanus* and *C. ligniperda* appears to have some likelihood, since seasonal and diurnal timing of swarming and the weather conditions do not differ significantly over all data known from the area of Central Europe. Direct observations of synchronic and syntopic flights are known from the Saxon sandstone mountains (Elbsandsteingebirge) where *C. herculeanus* and *C. ligniperda* nest in close neighborhood—the former at the cool northern flank of the steep sandstone rocks where also other montane floral and faunal elements are observed and the latter at the warm southern flank with the elements of warm lowland heath. However, no hybrids were observed so far even in this region. In *C. herculeanus*, the swarming season in Germany is, according to 34 observations, 12 June \pm 19 days (18 May–3 August), with 82% of swarms having occurred before 1 July. Ten observations of swarming were at 16:45 \pm 1:37 (14:22–19:00) h solar time and at air temperatures of 20–28 °C. The data for *C. ligniperda* are highly similar: 26 observations describe the swarming season as 5 June \pm 10 days (3 May–26 June) and 11 observations of swarming occurred at 16:19 \pm 2:39 (10:50–20:15)

h solar time and at air temperatures of 21–28 °C (all data from Seifert 2018).

This paper will describe the first detected hybridization case of *C. herculeanus* × *ligniperda* thanks to a sample discovered and kindly provided by the Danish myrmecologist Jörn Bittcher/Albertslund. Furthermore, I will give here improved data on the separation of workers of the parental species.

Materials and Methods

Material

High quality NUMOBAT data as the basis of this paper were recorded in a total of 61 nest samples and 140 worker individuals which are all deposited in the collection of Senckenberg Museum of Natural History Görlitz. This material is given below and is listed in the following sequence and format: site, date in the format yyyy.mm.dd, field sample number “field No” which is found on the mounted specimens (latitude in decimal format, longitude in decimal format, and meters above sea level). The accuracy of coordinates is proportional to the number of decimal points and “xx” in the sampling date sequence mean missing data. In some samples without any direct or derived information on date, the assumed period is given.

Camponotus herculeanus (Linnaeus, 1758)

A total of 26 nest samples with 72 workers were investigated.

Denmark: Hammer Bakker, 2018.08.14 (57.129, 10.033, 50). Finland: Renko—13 km SW, 1996.07.11, No 40 (60.836, 24.067, 136). Germany: NP Bayerischer Wald: Guglöd—1 km NNW, 2002.xx.xx (48.936, 13.424, 825); Carlsfeld—3.2 km S, 1979.07.01 (50.407, 12.589, 942); Görlitz, 2014.08.03 (51.143, 14.983, 228), Liebstein—2.0 km W, 1988.05.08 (51.201, 14.876, 240); Lömischau, 1961.06.05 (51.28, 14.57, 144); Niedercunnersdorf, 1910.04.16 (51.053, 14.684, 375); Oybin, 1919.09.02 (50.84, 14.75, 400); Ottersberg, 1998.07.xx (53.11, 9.14, 15); Schwarzburg, 1992.03.03 (50.64, 11.20, 280); Uhlstädter Heide 1987.xx.xx (50.70, 11.46, 360); Uhlstädter Heide 1988.xx.xx, No S1 (50.70, 11.46, 360); Uhlstädter Heide 1988.08.02 (50.70, 11.46, 360); Vordere Partschenhörner, 1969.07.xx (50.883, 14.300, 450); Waldbrunn-S, 1992.06.xx (49.44, 9.08, 460); Waltersdorf: Lausche, 1986.11.16 (50.849, 14.646, 776). Kazakhstan: Tian Shan: near Alma-Ata, pre 1970 (43.14, 76.90, 1400); Saur Mountains: Mata Gul, 2001.07.28 (47.055, 84.924, 1750). Kyrgyzstan: Sary Tschelek, 1998.07.21, No 080 (41.817, 71.967, 1290). Russia: Altai Mountains: Ortolyk—15 km NW, 2000.07.27, No 6 (50.117, 88.317, 1850). Slovakia: Vysoke Tatry, pre

1970 (49.13, 20.11, 1300); Vysoke Tatry, 1981.06.12 (49.21, 20.26, 1200). Sweden: Orsa-45 km N, 2002.08.04, No 038a (40.408, 14.820, 476); Öland: Byrums Sandvik, 1992.06.14 (57.07, 16.86, 10); Öland: Böda, 1992.06.14, No 135 (57.25, 17.06, 9).

Camponotus herculeanus × *ligniperda*

A nest sample with six workers from Denmark: Skagen: Tingmandsklit, 2018.05.05 (57.711, 10.516, 8) was investigated.

Camponotus ligniperda (Latreille, 1802)

A total of 34 nest samples with 62 workers were investigated.

Bulgaria: Stara Planina: Botev-S, 1985.07.20 (42.698, 24.931, 1480); Velingrad: Sveta Petka, 1983.07.14 (42.039, 23.876, 1240). Denmark: Tisvilde Hegn, 2014.04.16 (56.031, 12.025, 7). Finland: Nastola—3 km E, 1996.07.11, No 25 (60.930, 26.069, 126); Turku—22 km SSW: Sandvik, 1996.07.10, No 32 (60.242, 22.152, 20). France: Vitrac-s-Montane—1.8 km S, 2008.08.28, neotype of *Camponotus ligniperda* (45.360, 1.944, 550). Germany: Baruth, 1965.08.27 (52.235, 14.597, 180); Blösa, 1924.08.25 (51.15, 14.50, 242); Dauban, 1962.06.08 (51.284, 14.628, 157); Dresden: Heller, 1918.05.05 (51.105, 13.743, 170); Groß Saubernitz, 1962.09.12 (51.23, 14.64, 162); Groß Saubernitz, 1965.05.03 (51.23, 14.64, 162); Groß Saubernitz, 1966.07.20 (51.23, 14.64, 162); Großpostwitz: Mehlteuer, 1961.07.18 (51.140, 14.480, 366); Großschönau, 1970.03.31 (50.89, 14.66, 330); Halbendorf, 1967.05.22 (51.301, 14.549, 140); Klein Saubernitz, 1962.09.28 (51.264, 14.602, 150); Kümmitz-W, 2006.04.25 (51.893, 13.589, 76); Königshain, 1989.08.01 (51.198, 14.851, 300); Lömischau, 1966.07.02 (51.28, 14.57, 144); Meissen: Bosel, 1982.06.09 (51.138, 13.515, 170); Neukirch: Georgenbad, 1964.08.19 (51.082, 14.290, 350); Niedergurig: Gottlobsberg, 1961.05.05 (51.220, 14.470, 180); Niederoderwitz, 1919.xx.xx (50.95, 14.73, 310); Ober-Prauske, 1963.06.06 (51.25, 14.66, 193); Schwarza, 1988.xx.xx (50.676, 11.338, 286); Vordere Partschenhörner, 1969.xx.xx (50.883, 14.300, 450); Weigersdorf, 1962.10.02 (51.26, 14.64, 169); Weigersdorf, 1963.07.22 (51.26, 14.64, 169); Weigersdorf, 1964.06.20 (51.26, 14.64, 169); Weigersdorf, 1966.07.20 (51.26, 14.64, 169). Sweden: Blekinge: Aryd-3 km E, 1992.06.15 (56.211, 15.063, 25); Öland: Byrums Sandvik, 1992.06.14 (57.07, 16.86, 10); Valdemarsvik-W, 2007.06.13, No 003 (58.172, 16.854, 3).

Equipment and measurement procedures

All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations

around *X*, *Y*, and *Z* axes. A Leica high-performance stereomicroscope M165C equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) was used at magnifications of 120×–360×. A Schott KL 1500 LCD cold-light source equipped with two flexible, focally mounted light cables, providing 30°-inclined light from variable azimuth directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimum resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating the voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks was used. To avoid parallax error, its measuring line was constantly kept vertical within the visual field.

The morphometric characters and removal of allometric variance

Seven morphometric characters were investigated in worker ants. In bilaterally developed characters (HTL and SL), arithmetic means of both sides were calculated.

CL—maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of posterior vertex reduce CL.

CS—cephalic size; the arithmetic mean of CL and CW used as a less variable indicator of body size.

CW—maximum cephalic width; across, anterior or posterior of eyes, whichever yields the maximum measurement.

HTL—hind tibia length; with full width and length of hind tibia positioned in visual plane, measured from the most distal point near the extensor profile to the proximal constriction point of flexor profile (point A in Fig. 385 in Seifert 2018).

ML—mesosoma length from its caudalmost median point to its anteromedian margin. If the anterior fringe (or neck

shield) is concealed, measure to transition point between anterior pronotal slope and neck shield and multiply the value with 1.03.

PLG—mean length of pubescence hairs on dorsal part of first gaster tergite. Ten hairs are measured in the area demarcated by transverse lines positioned about 0.5 and 1.5 bandwidths frontal of the glossy terminal band. Considering this rule is important as hair length increases considerably more frontal of this area.

SL—maximum straight line scape length excluding the articular condyle.

sqPDG—square root of mean pubescence distance PDG on dorsal part of first gaster tergite in front of the glossy terminal band. Several counts along four transverse measuring lines variably positioned between 0.5 and 1.5 bandwidths frontal of the glossy terminal band are averaged until the sum of hairs counted is 40 at least. If *n* is the number of pubescence hairs crossing a measuring line of length *L*, mean pubescence distance is *L/n* and given in μm. Hairs just touching the line score as 0.5.

The enormous variance in absolute body size in connection with very strong allometries observed in most of the characters obscures interspecific differences of absolute values and primary shape variables. To reveal in comparative tables which shape variables do really differ between the species independent from body size and to improve the demonstration of hybrids, a removal of allometric variance (RAV) was performed with the procedure described by Seifert (2008). However, in contrast to this source, RAV was computed here by polygonal functions of the type $y = ax^2 + bx + c$, describing the mean of the species-specific functions of *C. herculeanus* and *C. ligniperda*. Polygonal functions achieved the same reduction of intraspecific variance as diphasic linear functions, but were preferred because of simpler description. RAV was calculated assuming all the individuals to have a cephalic size of CS = 2.5 mm. In the following functions, PLG and PDG have to be entered in μm and CS in mm.

$$CL/CW_{2.5} = CL/CW / (0.0297 \times CS^2 - 0.3072 \times CS + 1.5865) \times 1.0041$$

$$SL/CS_{2.5} = SL/CS / (0.0329 \times CS^2 - 0.3664 \times CS + 1.6264) \times 0.9160$$

$$HTL/CS_{2.5} = HTL/CS / (0.0344 \times CS^2 - 0.3815 \times CS + 1.7915) \times 1.0528$$

$$ML/CS_{2.5} = ML/CS / (0.0334 \times CS^2 - 0.3475 \times CS + 2.132) \times 1.4715$$

$$PLG_{2.5} = PLG / (-4.928 \times CS^2 + 29.0 \times CS + 41.377) \times 83.08$$

$$spPDG_{2.5} = sqPDG / (-0.0981 \times CS^2 + 1.422 \times CS + 5.472) \times 8.410.$$

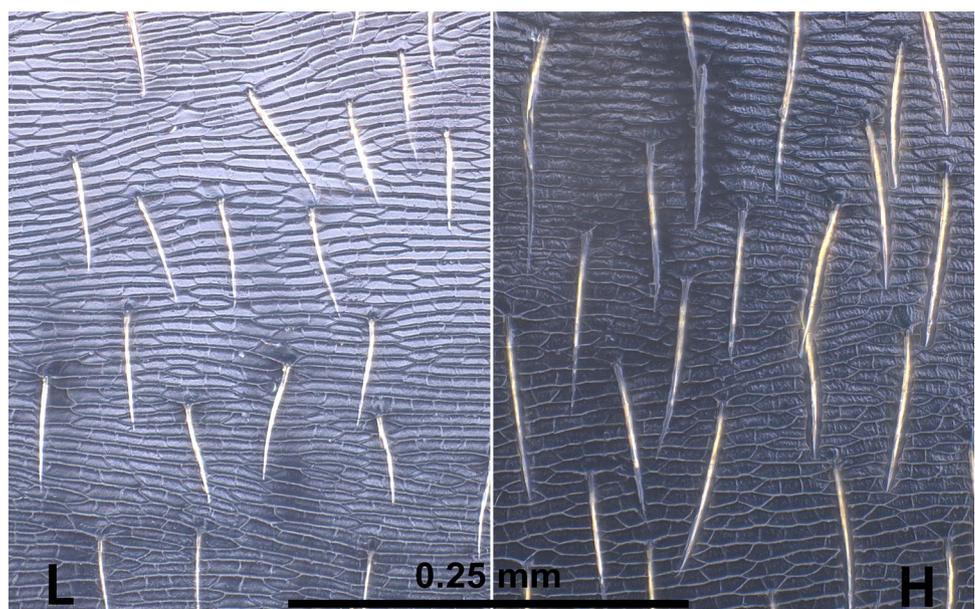
Multivariate analyses

Demonstration of heterospecificity of the parental species was done by several forms of exploratory data analyses: NC-Ward clustering, NC-part.hclust, the iterative vector-quantization method NC-part.kmeans, NC-NMDS-kmeans, principal component analysis (PCA) and linear discriminant analysis according to the procedures used by Seifert et al. (2013) and Csösz and Fisher (2015). Identification of hybrids was done by wild-card runs in a linear discriminant function (LDA) and by PCA.



Fig. 1 Hybrid worker of *Camponotus herculeanus* × *ligniperda* showing the dark wine red mesosoma suggesting a *C. herculeanus* heritage and a bright and large red patch on frons of gaster approaching the *C. ligniperda* condition

Fig. 2 Typical micro-structures of dorsal plane of first gaster tergite in *Camponotus herculeanus* (H, right) and *C. ligniperda* (L, left). *C. herculeanus* differ by much longer pubescence hairs and the presence of very delicate longitudinal micro-carinulae running between the larger transverse microripples



Results and discussion

Identification of hybrids

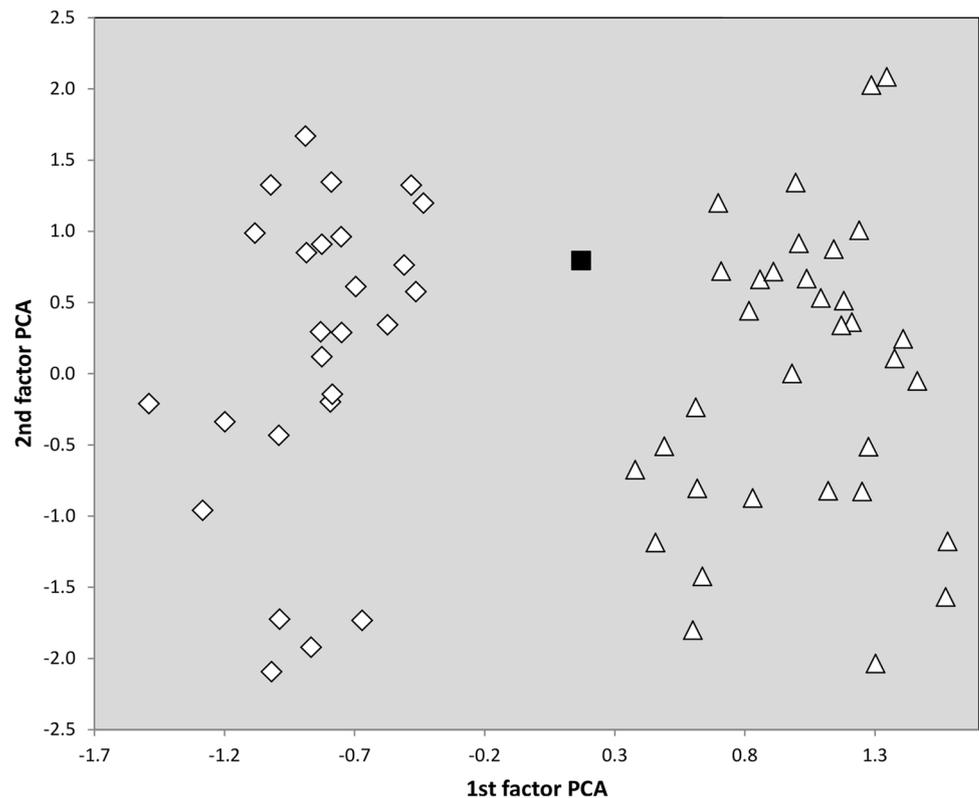
As described by Pisarski (1961) and Seifert (1989), pigmentation of mesosoma, petiole, and first gaster segment is variable in the two *Camponotus* species considered here. This refers to *C. herculeanus* in particular. A typical coloration in this species is a dark wine red mesosoma, a slightly lighter wine red petiole, and a patch of the same color at the frontal face of first gaster segment which varies in extension from slightly smaller to slightly larger than the caudal projection of petiole scale. Yet, very dark individuals with the promesonotum becoming blackish red to nearly black are not rare in large workers and, as the other extreme and more rarely occurring, medium-sized non-callow workers with lighter reddish mesosoma may occur in the same nest together with darker specimens. *Camponotus ligniperda*, typically showing a lighter reddish mesosoma and petiole and a big reddish area covering the whole anterior half of first gaster tergite, shows also some variation in brightness and extension of reddish pigmentation but less so than *C. herculeanus*. This variability precludes the use of pigmentation for species identification, but uncorrelated character expression may raise suspicion of a possible hybrid identity. This was the case in the Danish sample from Tingmandsklit, with some workers showing the dark reddish mesosoma pigmentation typical for *C. herculeanus* in combination with a more extended light red patch on first gaster tergite suggesting also a *C. ligniperda* parenthood (Fig. 1). This confusing impression was strengthened by structural characters such as pubescence and microsculpture on the first gaster tergite

Table 1 RAV-corrected (rows 2–7) and primary (rows 8–13) morphometric data of worker individuals of *Camponotus herculeanus*, *C. ligniperda*, and interspecific hybrids *C. herculeanus* × *ligniperda* given as arithmetic mean ± standard deviation (lower extreme, upper

extreme); n = number of individuals. F values and significance levels p are from a univariate ANOVA and evaluate the differences between *C. herculeanus* and *C. ligniperda*. CS is given in mm and sqPDG and PLG in μm

	<i>herculeanus</i> ($n=72$)	ANOVA $F_{1,132}, p$	<i>ligniperda</i> ($n=62$)	<i>herculeanus</i> × <i>ligniperda</i> ($n=6$)
CS	2.436 ± 0.658 (1.272, 3.537)	0.284, n.s	2.491 ± 0.523 (1.336, 3.428)	2.929 ± 0.328 (2.340, 3.257)
CL/CW _{2,5}	0.961 ± 0.020 (0.907, 0.996)	542.5, 0.000	1.049 ± 0.024 (0.982, 1.108)	1.022 ± 0.016 (1.009, 1.046)
SL/CS _{2,5}	0.883 ± 0.024 (0.820, 0.936)	206.0, 0.000	0.950 ± 0.030 (0.867, 0.998)	0.928 ± 0.014 (0.913, 0.945)
HTL/CS _{2,5}	0.977 ± 0.029 (0.918, 1.062)	825.1, 0.000	1.128 ± 0.032 (1.039, 1.189)	1.052 ± 0.014 (1.033, 1.072)
ML/CS _{2,5}	1.415 ± 0.033 (1.326, 1.482)	360.7, 0.000	1.526 ± 0.034 (1.400, 1.608)	1.465 ± 0.038 (1.418, 1.512)
sqPDG _{2,5}	7.65 ± 0.69 (6.09, 9.33)	150.2, 0.000	9.33 ± 0.89 (7.92, 12.75)	8.87 ± 0.39 (8.35, 9.52)
PLG _{2,5}	102.5 ± 11.4 (82.6, 134.7)	547.4, 0.000	64.0 ± 6.6 (49.1, 76.2)	91.8 ± 5.7 (83.5, 97.5)
CL/CW	0.982 ± 0.100 (0.848, 1.168)	20.4, 0.000	1.060 ± 0.097 (0.911, 1.242)	0.962 ± 0.048 (0.911, 1.046)
SL/CS	0.909 ± 0.135 (0.703, 1.171)	5.6, 0.019	0.961 ± 0.117 (0.756, 1.192)	0.849 ± 0.056 (0.786, 0.947)
HTL/CS	1.003 ± 0.136 (0.761, 1.272)	36.5, 0.000	1.139 ± 0.124 (0.945, 1.409)	0.972 ± 0.062 (0.904, 1.078)
ML/CS	1.439 ± 0.111 (1.229, 1.677)	25.2, 0.000	1.538 ± 0.116 (1.363, 1.778)	1.398 ± 0.077 (1.345, 1.543)
sqPDG	7.55 ± 0.77 (5.39, 9.57)	113.5, 0.000	9.30 ± 1.11 (7.81, 13.53)	9.26 ± 0.50 (8.90, 10.26)
PLG	99.7 ± 13.5 (76.6, 136.2)	389.4, 0.000	62.9 ± 6.3 (46.7, 72.6)	92.4 ± 5.8 (84.0, 98.2)

Fig. 3 Principal component analysis of seven NUMOBAT characters of *Camponotus herculeanus* (white rhombs), the hybrid *C. herculeanus* × *ligniperda* (black square) and *C. ligniperda* (white triangles). Nest sample means are shown. The first factor of PCA explains 68.8% of variance



which did not match the standards of the parental species (Fig. 2). Convincing evidence for hybrid identity of this sample is presented by the NUMOBAT data presented in the following paragraph.

Compared to the primary data, the removal of allometric variance (RAV) resulted in an enormous reduction of the mean intraspecific coefficient of variation in two-thirds of

the characters: it was reduced to 23% in CL/CW, 22% in SL/CS, 24% in HTL/CS, 30% in ML/CS, 84% in sqPDG, and 93% in PLG (Table 1). Exploratory data analyses on nest sample basis provided a very strong and error-free separation of *C. herculeanus* and *C. ligniperda* in any of the four NC-clustering methods, but none of these methods was able to expose the *C. herculeanus* × *ligniperda* sample as own

Fig. 4 Principal component analysis and linear discriminant analysis of seven NUMOBAT characters of *Camponotus herculeanus* (white rhombs), the hybrid *C. herculeanus* × *C. ligniperda* (black square) and *C. ligniperda* (white triangles). Nest sample means are shown. The individuals of the hybrid sample were run in the LDA as wild cards

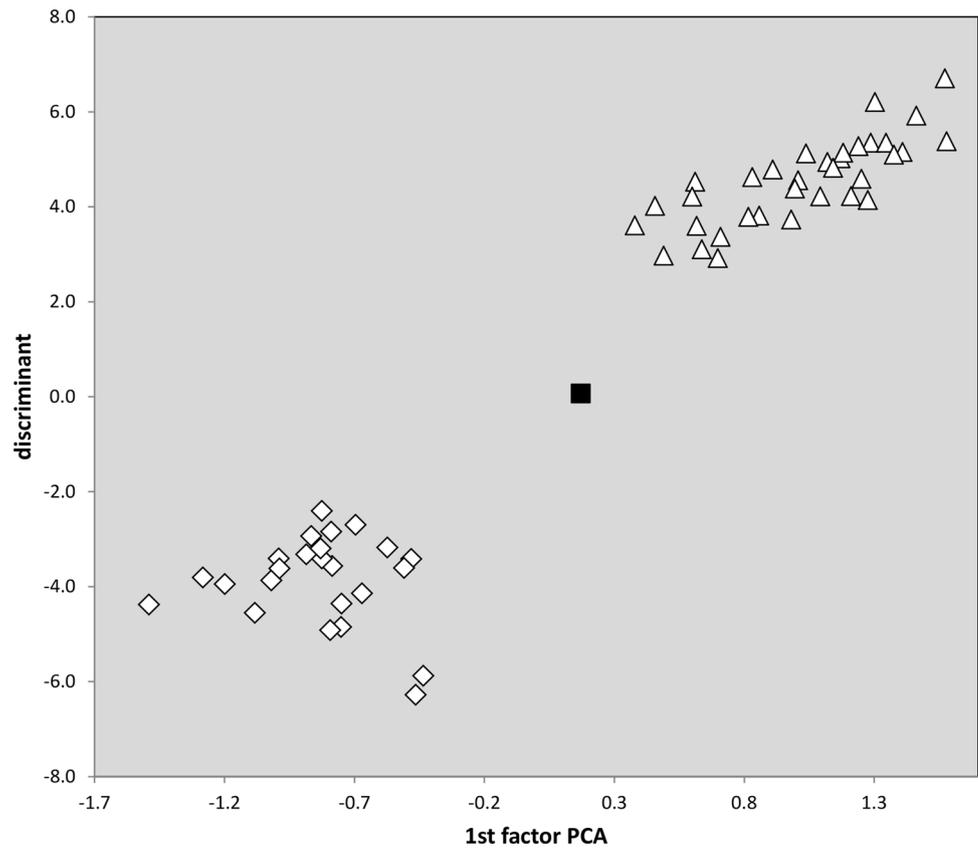
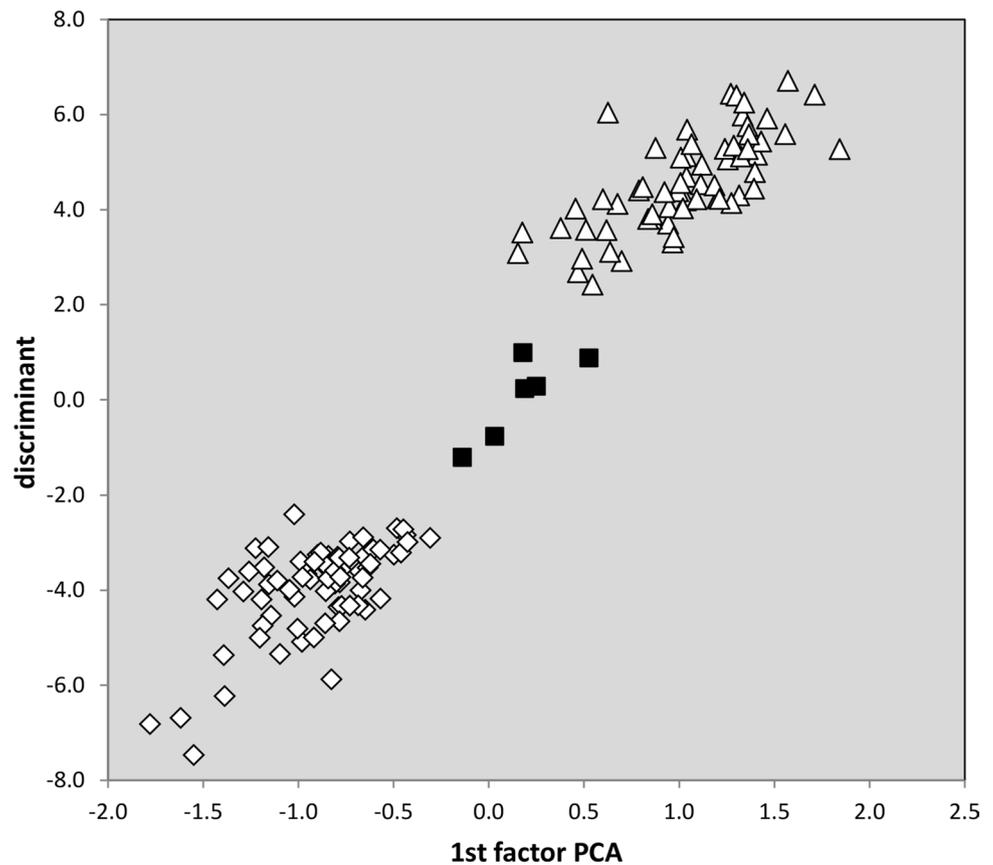


Fig. 5 Principal component analysis and linear discriminant analysis of seven NUMOBAT characters of *Camponotus herculeanus* (white rhombs), the hybrid *C. herculeanus* × *C. ligniperda* (black squares) and *C. ligniperda* (white triangles). All individuals of the hybrid sample were run in the LDA as wild cards



entity: the Tingmandsklit sample was placed by NC-Ward clustering within *C. ligniperda* and by NC-part.hclust, NC-part.kmeans, and NC-NMDS-kmeans within *C. herculeanus*. Yet, the principal component analysis (PCA) clearly exposed the intermediate position of the hybrid sample (Fig. 3). If all workers of this sample are run as wild card in a LDA, the sample mean is placed well separate from and exactly intermediate between the clusters of parental species (Fig. 4), whereas any sample of parental species is not placed intermediate if being subject to this procedure. The intermediate position of the hybrid sample is here shown even on the level of individuals (Fig. 5). In contrast, the hybrid individuals are not exposed when a PCA is run with primary data (absolute measurements), because the separation of the parental species is too weak.

Isolated consideration of the expression of single characters in the Tingmandsklit sample shows a heterogeneous, fluctuating picture as is typical for hybrids. RAV-corrected data of CL/CW_{2.5} and SL/CS_{2.5} approach the condition in *C. ligniperda* and of PLG_{2.5} that of *C. herculeanus*, whereas sqPDG_{2.5}, HTL/CS_{2.5}, and ML/CS_{2.5} are nearly intermediate (Table 1).

Data without RAV show a huge overlap between the parental species and provide no suggestion of the true identity of the hybrid sample. A comment is necessary to the CS data in Table 1. Precise calculation of allometry functions required to include enough individuals near the lower and upper extremes of body size. As a consequence, the CS data distribution in Table 1 is an artefact of biased size selection, showing a much larger coefficient of variation than it would result from random sampling in nature.

It may be asked if there are other factors apart from hybridization which could cause the described morphological intermediacy. First of all, there are no examples known in ants of phenotypes intermediate between two species when the genome of only a single species is involved. On the other hand, there are several cases in animals that F₁ hybrids of two species A and B may represent phenotypic copies of a third species C. A classical example from ornithology (Grant et al. 2000) is the F₁ hybrid of the ducks *Aythya fuligula* (Linnaeus 1758) and *Aythya ferina* (Linnaeus 1758) which are highly similar to non-hybrid specimens of *Aythya affinis* (Eyton 1838). The hypothesis that the Tingmandsklit sample might represent a third so far undescribed species with an intermediate phenotype can be rejected as these huge, impressive ants have always attracted the attention of myrmecologists since the eighteenth century and the finding of only one case within hundreds of samples of a well-studied geographic region speaks against the existence of a true population of a third species.

This first established hybrid case raises the question how frequent hybridization in this species pair really is. Investigation of some 500 samples from Europe by subjective

eye inspection or making a few simple measurements as required by the keys of Seifert (1989) or Seifert (2018) did not raise suspicion of further hybrid cases, but it cannot be excluded with certainty that a few further cases escaped my attention in the past during these quick examinations. Considering this, the ratio of hybridization in the sympatric range is assumed to range between 0.2 and 1%. Taking into account the very large overlap of swarming times and syntopic occurrence in an estimated 10% of the known sites, the low hybrid frequency indicates strong reproductive barriers between the species.

Fixation of a neotype of *Camponotus ligniperda* (Latreille 1802)

Latreille spent unusually much space arguing about “la torture pour les reconnoitre” of the two huge red-breasted ant species named, at that time, *Formica ligniperda* and *Formica herculeana*. There is one statement in the description that suggests non-synonymy of his ant with *Camponotus herculeanus*: “L’abdomen est...noir, luisant, avec le devant du premier anneau d’un rouge sanguin,…” (The abdomen is black, shining, with the anterior part of the first ring blood-red). This wording does not clearly quantify how large the red surface in front of the first gaster segment really was, but Latreille most probably meant a larger patch of a lighter red that is typical for *Camponotus ligniperda* (Seifert 1989, 2018). This vague indication gets some support from the climatic and geographic conditions at the type locality Brive-la-Gaillarde (45.17°N, 1.53°E, 115 m), the surroundings of which hardly exceed an elevation of 500 m. If we subtract 1.0 °C of global warming from the current climate data of Brive, the mean air temperature from 1 May to 31 August should have been 17.6 °C around the year 1800; this value decreases to 15.0 °C at elevations of 500 m, and the annual precipitation was about 700 mm. These data are within the optimum of the climate niche of *C. ligniperda*, but represent more marginal conditions within the niche of *C. herculeanus* (Seifert 1989, 2017, 2018). Accordingly, we may expect *C. herculeanus* to have been much rarer or absent in this region during Latreille’s time.

Whatever interpretation is given, there is no definite proof which ant Latreille really meant and investigation of type specimens is required. However, according to a message from J. Casevitz-Weulersse of 13 June 2008, there are no specimens in the collection of Muséum National d’Histoire Naturelle Paris that could be considered as types. To finally settle the identity of *Camponotus ligniperda*, a neotype is fixed herewith at a medium-sized worker labelled “FRA: 45.3600°N, 1.9444°E/Vitrac-sur-Montane 1.8 km S/550 m, leg. Galkowski 2008.08.28” and “Neotype *Camponotus ligniperda* (Latr. 1802) des B. Seifert 2018”. This specimen was collected with two other workers from a nest found

38 km NE of Brive-la-Gaillarde and is stored in the SMN Görlitz.

A comment on Latin naming of *Camponotus ligniperda*

Two Latin spellings of the ant which Latreille had also named in French “fourmi rongé-bois” have been in use by various authors over the last 60 years: *Camponotus ligniperda* and *C. ligniperdus*. This disparity causes confusion (but is in reality not a very important issue because it does not lead to confusion with other *Camponotus* species). The latter spelling assumes that “*ligniperda*” is a female adjective attached to the female noun *Formica* and has to change its ending to “-us” when the species is transferred to the masculine genus *Camponotus*. The other spelling assumes that Latreille used “*ligniperda*” as a noun in apposition, which remains unchanged in combination with a genus name of any gender (§ 31.2.1. and § 31.2.2. of ICZN). This interpretation as a noun (meaning “wood destroyer”) was clearly expressed by Kutter (1977) and I consistently followed this usage throughout the last 40 years. Indirect conclusions on Latreille’s naming intention, considering the vernacular compound word “rongé-bois”, appear problematic as I received different proposals by native French speakers. The deciding point in this debate is that “*ligniperda*” is no accidental linguistic fault—this word really exists as a Latin noun and Pierre André Latreille, as a Latin-educated catholic priest, and Heinrich Kutter, as an old-school pharmacist educated in the 1920s, should have known this. To have this view confirmed, I asked the distinguished Latin expert Prof. Thomas Baier from the Institute of Classical Philology of the University of Würzburg. He fully concurred. This is what he wrote in a letter of 15 July 2013: “... assessing your problem according to the rules of classical Latin, *ligniperda* would be a noun, which always is written *ligniperda* in connection with masculine and feminine, thus not being adapted in its suffix—just as you have assumed. A parallel form is *parricida* (murderer of relatives). Johann von Schwaben, who killed his uncle Albrecht von Schwaben around 1300, is known since then in the history books (and in Schiller’s *Wilhelm Tell*) as “*Johannes parricida*”. What applies to Swabian dukes also applies to ants, *si parva licet componere magnis*...”.

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